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Biochemical Analysis of Different Varieties of Honey Samples

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Abstract

Honey is a sweet and viscous substance made by several bees, the best-known of which are honey bees. The major components of honey are sugars i.e., fructose and glucose (monosaccharides) constitute around 70% of the total, the disaccharides such as sucrose constitute 10% and 17 to 20% is water, which is found in dissolved form. Honey is also known for its flavor, aroma, and color, also contains 181 different substances. The composition of honey depends on the plant sources from which the nectar is collected, and also on the soil, weather, and other factors, hence its composition varies from one honey to another. In this paper, we will discuss about biochemical properties of different varieties of honey samples by determining their physical properties like color, acidity, or pH and chemical properties like glucose, ascorbic acid, proteins, and amino acids.

Keywords: *Color, Acidity or pH, glucose, ascorbic acid, proteins, and amino acid.*

1. Introduction

The major components of honey are sugars i.e., fructose and glucose (monosaccharides) constitute around 70% of the total, the disaccharides such as sucrose constitute 10% and 17 to 20% is water, which is found in dissolved form. Honey is also known for its flavor, aroma, and colour, also contains 181 different substances. The composition of honey depends on the plant sources from which the nectar is collected, and also on the soil, weather, and other factors, hence its composition varies from one honey to another [Doner, 1977]. As honey consists of around 80% of sugar it tastes sweet and this sweetness varies as the sugars differ in their sweetness. Fructose and glucose are the main sugar derivatives found in all the honey in almost equal proportions and this proportion varies from honey to honey. Glucose is less sweet compared to sucrose, and sucrose is less sweet compared to fructose. The honey should be stored in suitable conditions. If not, there are chances of occurrence of fermentation in honey [Bogdanov et al., 2008]. There are mainly four different types of honey bees namely- Apis dorsata (Rock bee), Apis indica (Indian bee), Apis Serena (Asian bee), Apis melliifera (European bee). A. dorsata and A. Serena are furious, A. florea is mild, and A. mellifera is gentle except African subspecies. A. dorsata produces 40 kg honey per colony and is the biggest honey bee which measures around 16 to 18 mm. A. florea can produce 500 g of honey per colony and is the smallest bee measuring around 10mm. A. cerena produces 5 kg honey per colony, measuring around 14 to 15 mm. A. mellifera is a medium-sized bee producing 15 kg honey per colony and is of medium-sized bee measuring around 14 to mm. The honey bee colony shows the division of labor, where the entire colony is divided into queen, worker, and drone. Only one queen can be found in a single colony and will produce all the workers and drones by acting as a mother of the whole colony, which lays around 1500 to 2000 per day [1-5]. The

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queen will release a pheromone (Queen substance) which maintains the colony's organization, in the absence of the queen pheromone the colony will start building a new queen thinking that the old queen is lost. Workers are the females who cannot be able to mate, their work is to clean the hive, raise all queens by producing royal jelly whenever they need, hive ventilation, guarding the hive, bee wax secretion, and conversion of nectar to honey. Drones are fertile males; they don't have any particular work inside and outside the hive. They will be only produced during the breeding season. After the mating, they have been driven out of the colony or each drone can be eaten by 3 to 4 worker bees. [A TEXT BOOK OF APICULTUR- Dr. J.K. Gupta, Dr. V. V. Belavadi, Sh. Mohinder Singh]. Honey bees and flowers can be taken as an example of mutualism and co-evolution [Crane, 1990]. Honey bees act as a bioindicator of nature and belong to hymenopterans, which means they only feed on plant sources like nectar and pollen fully. Honey is considered a symbol of prosperity and sanity. Honey can be defined as nectar that has been modified, stored, and shielded in the well of the combs by the honey bee. Honey also contains or acts as an antiseptic, antibiotic, and antioxidant and it is an ingredient of many pharmaceuticals, Bakery, Cosmetics, confectionery, and also in the tobacco industry. The honey quality depends on the total amount of nectar secreted in the plant and also the concentration of sugar in the nectar [Seema Singh Thakur and Sudha Kanauja, 2003]. Honey contains many components like fructose [19%] glucose [31.28%] sucrose [1.31%] disaccharides like maltose [7.31%], moisture [17.2%] highest Sugars [1.5%], free acids as gluconic [0.43%], lactose as gluconolactone [0.14%], Ash [0.16%] and nitrogen [0.041%] – [Jeffrey and Echazarreta, 1996], also contain a little number of fatty acids like palmitic [16:0] oleic [18:1] and linoleic [18:3] – [Tan et al., 1988; Singh and Kaur Bath, 1997].

2. Materials and Methodology

2.1 Materials

Seven honey samples were collected from different regions of Ramanagar, Hassan, Tumkur, and Bengaluru rural districts. Of which 3 samples were of Apis mellifera, 2 samples were Apis dorsata, 1 sample of Apis florea, and 1 sample of Apis cerana indica (Table 1). The honey samples were coded with sample numbers 1 to 7. A commercially available honey was taken to compare the readings, and this sample was coded as sample number 8, The collected honey samples were stored in dry and ambient temperature before the analysis was performed [6-8].

Table 1 Sample

Sample A	*Apis mellifera*
Sample B	*Apis mellifera*
Sample C	*Apis mellifera*
Sample D	*Apis florea*
Sample E	*Apis dorsata*
Sample F	*Apis dorsata*
Sample G	*Apis cerena indica*
Sample H	Commercial honey

3. Methodology

3.1.Physical Properties

1. pH

10g of different honey samples were dissolved in 50 ml of distilled water, and the pH was checked using a pH meter [10-13].

2. Acidity

The total acidity was determined by the Titrimetric method; the addition of 0.05 N NaOH was stopped at pH 8.50 (free acidity), and immediately, a volume of 10 ml of 0.05 N NaOH was added and without delay back titrated with 0.05 M HCl from 10 mL to pH 8.30 [lactonic acid]

3. Colour intensity

The honey samples were diluted to 50% [w/v] with warm [45–50 °C] milli Q water and the solution was filtered through a 0.45 mL filter. There was a complete absence of coarse Particles in the honey solutions as all the commercial samples were non-

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crystalline liquid honey. The absorbance was measured using a spectrophotometer at 450nm [14].

3.2.Chemical Properties

1. Glucose

The glucose content is calculated using the titrimetric method [AQAC-Association of Official Analytical Chemists, official methods of analysis. In K. Helrich [Ed.] [15th ed.]. Arlington, VA, USA, Inc,1990]. 1 gm of honey was dissolved in water and then the volume was made up to 250mL. 50 mL of honey solution was taken from this in a stoppered flask, and to this 40 mL of iodine solution and 25 mL of sodium hydroxide solution were added. The flask was stopped and kept in the dark for 20 minutes. It is then acidified with 5 ml of concentrated sulphuric acid and quickly titrated the excess of iodine against standard sodium thiosulphate solution. By using 50 ml of water a blank was prepared and was run in place of honey solution [15-17]. The glucose content is calculated by using the formula,

$$\% \text{ of Glucose}(W) = (B-S) \times 0.004502 \times 100/A$$

B = Volume of Sodium thiosulphate solution required for the blank.

S = Volume of Sodium thiosulphate solution for the sample

A = Mass of honey taken for testing

2. Ascorbic acid

The ascorbic acid content is calculated by using the Indophenol method [Suzanne Nielsen] -Department of Food Science, Purdue University, West Lafayette, IN, USA. 0.5 g of honey was dissolved in 5 ml of water and this solution was titrated against the indophenol dye. The ascorbic acid content is calculated using the formula [18].

$$\text{Ascorbic acid} = (X - B) \times (F / E) \times (V / Y) \text{ mg/mL}$$

Where:

X = mL for sample titration

B = average mL for sample blank titration

F = Titre of dye (= mg ascorbic acid equivalent to 1.0 mL indophenol standard solution)

E = mL assayed (=2 mL)

V=volume of initial assay solution (=7 mL)

Y=volume of sample aliquot titrated (=7 mL)

3. Protein

A honey sample weighing one gram was dissolved in water and the volume is made up to 10mL. 1 mL of this solution was pipetted out into the test tube. 0.2,0.4, 0.6,0.8, and 1 ml of the working standard were pipetted out into the series of labeled test tubes. The volume made up to 1ml in all the test tubes using distilled water. A tube with 1 mL of distilled water served as the blank, then 5mL of alkaline copper reagent was added to all the test tubes, and the contents were mixed by vertexing/shaking the tubes and allowed to stand for 10 minutes. 0.5 mL of diluted Folin's reagent is added to all the test tubes and incubated at room temperature in the dark for 30 minutes. The absorbance is recorded at 660nm against the blank, then the graph was plotted by taking the concentration of protein along the x-axis and absorbance at 660nm along the y-axis. The protein values of honey samples were calculated using the standard graph [19-21].

4. Amino acid

Different honey sample weighing one gram was dissolved in warm water and volumes made up to 10 mL, take 1ml of the solution, were pipetted out different volumes(0.1ml-1ml) of standard amino acid solution to the respectively labeled test tubes added distilled water in all the test tubes to make up the volume to 4mL. Add 4 ml of distilled water to the test tube labeled blank. Now add 1ml of ninhydrin reagent to all the test tubes including the test tubes labeled 'blank' and 'unknown'. Mix the contents of the tubes by vertexing/shaking the tubes. Cover the mouth of the tubes with aluminum foil. Place all the test tubes in a boiling water bath for 15 minutes. Cool the test tubes in cold water add 1ml of ethanol to each test tube and mix well [22-25]. Now record the absorbance at 570 nm of each solution using a colorimeter then plot the standard curve by taking concentration along the x-axis and absorbance at 570nm along the y-axis.

4. Results

4.1 Physical Properties

1. pH

The pH of honey samples ranged from 4.27 to 4.85[26]. Among all the 8 samples, sample B has more pH content whereas sample A has less pH content (Figure 1).

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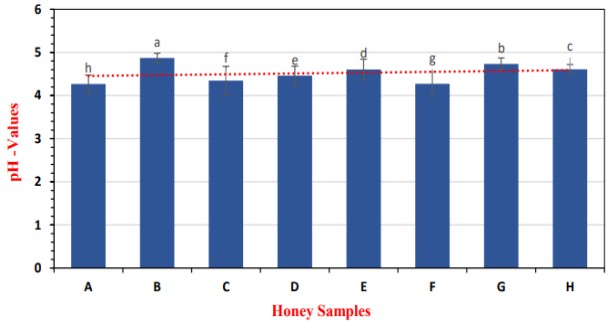

Figure 1 pH

2. Color Intensity

The color intensity of honey samples ranged from 0.24 to 1.13 mAU. Among all the 8 samples sample E has more color intensity, whereas sample A has less color intensity (Figure 2).

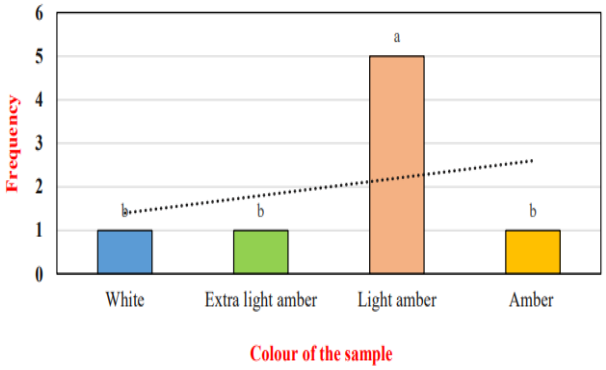

Figure 2 Color Intensity

3. Acidity

The acidity of honey samples ranged from 0.026 to 0.15 meq/kg. Among all the 8 samples, sample A has more acidity whereas sample G has less acidity content as shown in Figure 3 [27-29].

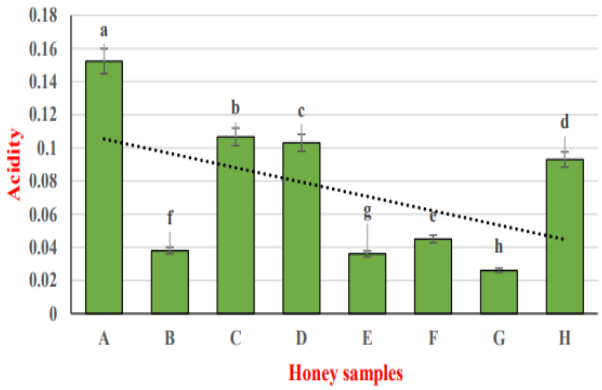

Figure 3 Acidity

4.2 Chemical Properties

1. Glucose Content

The glucose content of the honey samples ranged from .09 mg/mL to 1 18 mg/ml. among all the 8 samples, samples A, D, F, and G have more Glucose content whereas samples C and E have less glucose content as shown in Figure 4.

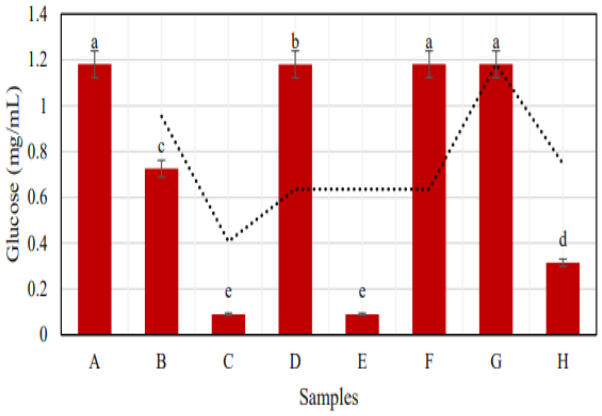

Figure 4 Glucose Content

2. Ascorbic Acid

The ascorbic acid content of the honey samples ranged from 0.5 mg/mL to 1.5mg/mL. Among all the 8 samples sample H has more ascorbic acid content, whereas samples B, E, and G have less ascorbic acid content as shown in Figure 5.

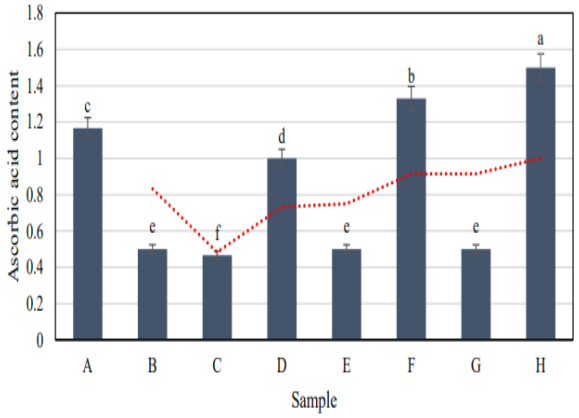

Figure 5 Ascorbic Acid

3. Protein Content

The protein content of the honey samples ranged from 45.893mg/mL to 223.23mg /mL (Figure 6). Among all the 8 samples sample H has more protein content whereas sample A has less protein content [30-31].

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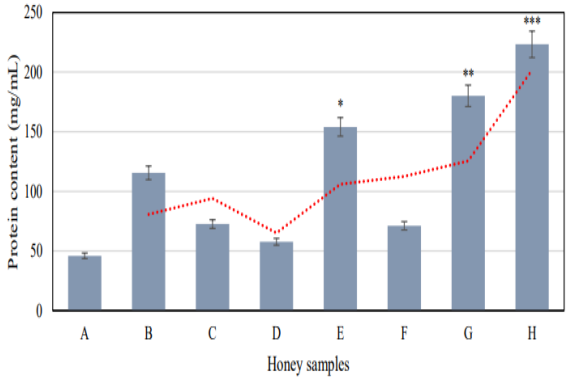

Figure 6 Protein Content

4. Amino Acid Content

The amino acid content of the honey samples ranged from 2.6mg/mL to 6.275 mg/mL (Figure 7). Among all the 8 samples, sample B has more Amino acid content whereas sample A has less Amino acid content [32].

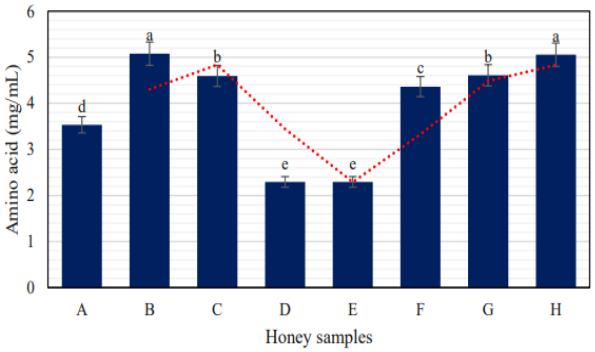

Figure 7 Amino Acid Content

Conclusion

Honey's color, color intensity, pH, acidity, and protein content are multifaceted attributes that collectively define its uniqueness. The color and color intensity of honey offers visual cues to its flavor and potential health benefits. The pH level and acidity play a crucial role in preserving honey and enhancing its taste The results of this study indicated that the physiochemical characteristics of fresh honey samples were within recommended limits of international standards than branded samples. Evidence showed that the freshness and purity of fresh honey were due to dominant flora. However, the honey is primarily a result of the nectar sources visited by bees [32-34]. The biochemical properties of the honey also depend on the flora they feed; it changes from region to region. Light-colored honey, such as clover or acacia honey, is often milder in flavor, while darker varieties like buckwheat or manuka honey tend to have more pronounced and robust taste profiles. The color of honey can also change over time due to factors like temperature, storage conditions, and the presence of impurities. A darker Honey might indicate a higher content of antioxidants and potential health benefits.

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